PERGAMON

Vision Research 41 (2001) 1885–1889

Vision Research

www.elsevier.com/locate/visres

Refractive state and accommodation in the eyes of free-swimming versus restrained juvenile lemon sharks (*Negaprion brevirostris*)

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Received 1 August 2000; received in revised form 15 November 2000

Dedicated to the memory of our friend and colleague, Dr. Perry Gilbert.

Abstract

Optical measurements of the refractive state of the eyes of various shark species typically have depicted sharks as hyperopic (far-sighted) with little evidence of accommodation (i.e. the ability to change focus for visualizing objects at different distances from the eye). In this study, we used infrared video retinoscopy to measure the refractive state in juvenile lemon sharks (*Negaprion brevirostris*). This technique allows dynamic measurement of refractive state in free-swimming animals as they pass by an aquarium window. We found that unrestrained lemon sharks are focused emmetropically relative to a 1-m distant photorefractor for the lateral visual field. However, when restrained either right side up or upside down (the latter inducing tonic immobility), the sharks become increasingly hyperopic, an artifact also reported in some other vertebrates. In addition, unrestrained lemon sharks display small amplitude accommodative excursions. Thus, refractive state measurements on restrained sharks in general may not reflect the natural, resting state of the shark eye, but rather, an induced hyperopia and lack of accommodative function. Such an artifact may be present in other vertebrate species, underscoring the need to obtain measurements of refractive state in unrestrained animals.

Keywords: Shark; *Negaprion brevirostris*; Eye; Vision; Optics; Refractive state; Accommodation; Retinoscopy; Restraint; Stress; Hyperopia

1. Introduction

Few investigations have been conducted on the physiological optics of elasmobranchs (sharks, skates and rays). Beer (1894) was the first to report slight hyperopia in the group. Subsequent studies by Franz (1905, 1931) and Verrier (1930) addressed accommodative changes in the eyes of a number of elasmobranchs, with both workers reporting consistent findings of hyperopia in these species. These studies led to the characterization of elasmobranch eyes as being hyperopic, with accommodation occurring, if present, for near vision (Walls, 1942; Duke-Elder, 1958; Gilbert, 1963).

Later work using retinoscopic or ophthalmoscopic measurements of refractive state in shark eyes continued to demonstrate hyperopia. Sivak (1974) reported refractive errors of +8.00 to +9.00 D in the lateral visual field of juvenile lemon sharks (*Negaprion brevirostris).* Dividing these measurements by 1.34 to correct for retinoscopy of eyes viewed in air through the wall of an aquarium (Hueter & Gruber, 1980) yields hyperopic errors of +6.0 to +6.7 D. Hueter (1980, 1991) also found retinoscopically measured hyperopias averaging +7.5 D (already corrected) in the same species, which was inconsistent with the lower

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PII: S0042-6989(01)00064-5

level of hyperopia (+2.76 D) resulting from a schematic eye of the juvenile lemon shark eye (Hueter 1980, 1991; Hueter & Gruber, 1982).

Spielman and Gruber (1983), using an aquatic contact lens system to refract large sharks out of water, reported uncorrected hyperopias of +10 to +12 D (+7.5 to +9.0 D corrected) on two adult lemon sharks. Other shark species refracted by retinoscopy or ophthalmoscopy have included the sandbar shark, *Carcharhinus plumbeus* (0 to +5.2 D corrected; Sivak & Gilbert, 1976), bull shark, *C. leucas* (+7.5 to +8.2 D corrected; Spielman & Gruber, 1983), Caribbean reef shark, *C. perezi* (+3.0 to +6.0 D corrected; Spielman & Gruber 1983), tiger shark, *Galeocerdo cuvier* (+0.8 to +6.0 D corrected; Spielman & Gruber, 1983), nurse shark, *Ginglymostoma cirratum* (−1.9 to +8.4 D corrected; Sivak & Gilbert, 1976) and spiny dogfish, *Squalus acanthias* (+3 to +6 D, corrected; Sivak, 1978). These various studies included measurements after anesthesia and pharmacological or electrical attempts to induce accommodative changes.

In all of these previous studies, experimental animals were restrained either in or out of the water in order to measure refractive error. Strong evidence of accommodative capability under these conditions was found only in the nurse shark after anesthesia (Sivak & Gilbert, 1976) and has never been demonstrated in any shark species without the use of drugs or electrical stimulation of the oculomotor nerve. Because sharks under natural conditions are free-swimming animals that typically require large oceanic areas for their normal behavior, we hypothesized that restraining these animals may have affected their refractive states under experimental conditions. The consistent measurements of hyperopia and lack of accommodative changes suggested that the stresses and/or exhaustive exercise associated with experimental animals being captured, handled and restrained may have biased these measurements. To investigate this possibility, we used a different approach to determine the refractive state of juvenile lemon sharks. This method allowed us to compare the refractive states of free-swimming sharks with those obtained from the same animals under restraint.

2. Methods

We employed infrared video photoretinoscopy (Schaeffel, Farkas, & Howland, 1987) to record the plane of focus of five free-swimming juvenile lemon sharks and four of the same sharks while restrained. Three of the sharks we examined were females and two were males, ranging in size from 73 to 79 cm total length. Eye diameter of this size range of juvenile lemon sharks is ≈14–19 mm (Hueter, 1980).

Each shark was allowed to swim freely in a 1800-l natural seawater tank, which had a flat, clear acrylic window along one side, at Mote Marine Laboratory, Sarasota, FL. The sharks were allowed at least 15 min after handling to acclimate to the tank under dim light conditions prior to each experiment. They were then videotaped with a Canon CCD black and white, infrared (IR) sensitive camera (Model CI-20R), using the 1/1000 s shutter setting and a Sony Watchman 8 mm videotape recorder. The sharks slowly swam in a 2 m-diameter circle inside the tank and interpretable reflexes were obtained only as the sharks swam adjacent to the window in the field of the camera. Each unrestrained animal was repetitively refracted in this fashion for a minimum of 20 min and a maximum of 2.5 h. A similar technique has been used to obtain reflexes from free-swimming sea otters (Murphy, Bellhorn, Williams, Burns, Schaeffel, & Howland, 1990) and cuttlefish (Schaeffel, Murphy, & Howland, 1999).

The photoretinoscopic procedure was similar for restrained sharks, except that the restraint allowed us to use neutralizing photoretinoscopy with ophthalmic lenses. In this procedure, sharks were manually restrained underwater either right side up or upside down, the latter inducing tonic immobility (Watsky & Gruber, 1990; Henningsen, 1994). The shark's eye being refracted was positioned close to the acrylic window, while concave or convex lenses were placed adjacent to the window on the air side until neutralization of the reflex was obtained. As the refraction was visible on the monitor screen, a consensus of the direction of the reflex by several observers was often obtained. The final refraction relative to the camera was taken either as the lens which provided no motion of the reflex, or the average of the lenses which gave the first positive

Table 1
Examination of restrained sharks[a]

Shark	Sex	Duration of exam (min)	No. of observations	Mean refraction (Diopters)	S.D.
II	F	30	11	1.76	2.86
VII	M	15	9	3.69	2.79
VIII	F	22	16	3.75	2.35
IX	M	15	10	3.43	1.65

[a] Average over all observations: 3.46 ± 2.11 Diopters.

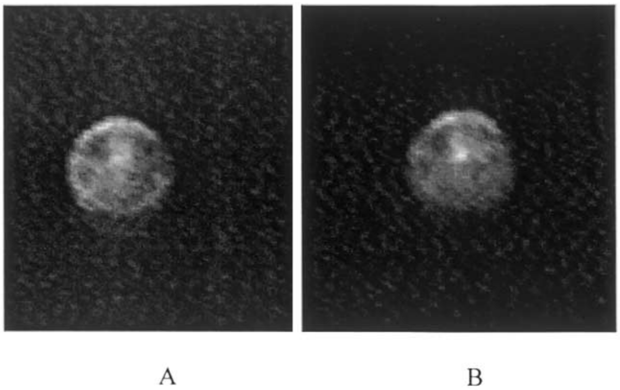

Fig. 1. Two infrared photoretinoscopic refractions of the eye of a free-swimming female juvenile lemon shark. These pictures are from a videotape recording of a shark taken ≈ 20 s apart as it swam by the camera. Note the relatively even lighting of the pupil (relative to the vertical axis) indicating that the shark is focused at the plane of the camera 1 m distant. If the refraction is myopic relative to the camera, more light will be seen at the bottom of the pupil; contra wise, if the refraction is hyperopic relative to the camera, more light would be seen at the top of the pupil. The unevenness of the illumination is largely due to the aberrations of the shark's eye.

indication of 'with' and 'against' reflexes around the neutral point. (The sex of the sharks, the durations of examinations and the number of observations are given in Table 1). We also performed conventional streak retinoscopy under the same conditions to confirm the results from photoretinoscopy. Unless otherwise stated, all refractions were corrected for the difference in refractive index of air and seawater (Hueter & Gruber, 1980) and for the working distance (i.e. refractions were expressed relative to infinity).

All experimental procedures were approved by Mote Marine Laboratory's Institutional Animal Care and Use Committee.

3. Results

All free-swimming lemon sharks were observed to be approximately emmetropically focused relative to the camera at a 1 m distance (Fig. 1). The sharks also were observed to make slight hyperopic or myopic refractive excursions while swimming past the window. Because the sharks were constantly moving, we could not neutralize these reflexes; however, on the basis of our experience with artificial eyes and the eyes of many other species, we estimated these excursions to be less than ± 1.5 D.

The refractions obtained from restrained animals differed significantly from those of free-swimming animals. Typically, the sharks were near emmetropic when first restrained, but became markedly hyperopic within a few minutes (Figs. 2 and 3, Table 1), with the degree of hyperopia increasing with time of restraint. There was no detectable difference in refractive state between animals held right side up (not in tonic immobility) or upside down (in tonic immobility). When a restrained shark was turned over and measured in the opposite orientation, however, there was an initial tendency of the animal to return briefly to near emmetropia, but then drift gradually into a hyperopic state again. For both orientations, the mean of the most ametropic refractions of all four restrained animals was $+6.3 \pm 1.2$ D (S.D.) and the maximum hyperopia we measured in a restrained shark was $+7.5$ D (corrected). The average ametropia (here, hyperopia) over all observations of the four restrained sharks was 3.46 ± 2.11 D.

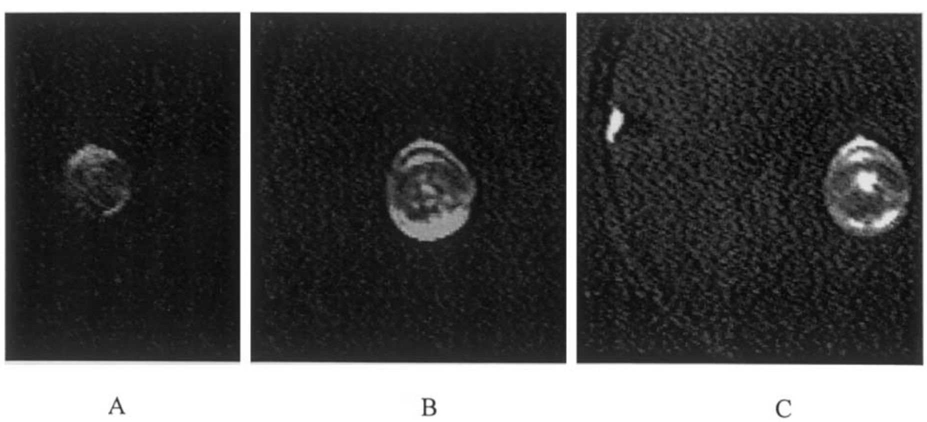

Fig. 2. Three sequential photoretinoscopic refractions of a restrained female juvenile lemon shark taken at 1 m distance. (A) No lens is in front of the eye, which is hyperopically focused (note light at top of pupil). (B) A $+12$ D lens is placed in front of the eye, which now is myopically focused relative to the camera (note light at bottom of pupil). (C) A $+7$ D lens is placed in front of the eye. The light is approximately evenly distributed in the pupil and the eye is in focus at the plane of the camera 1 m distant. This eye, therefore, is $+4.5$ D hyperopic (corrected for working distance and air–water interface) in this restrained shark. Again, the unevenness of the illumination in (C) is due largely to the monochromatic aberrations of the shark's eye.

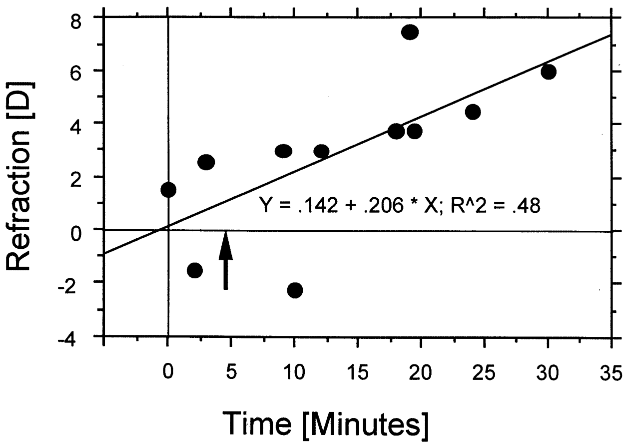

Fig. 3. Sequential refractions made with neutralizing photoretinoscopy of shark while restrained under water in an aquarium. First three measurements are of shark while right side up. Arrow indicates time at which shark was inverted. Over-all trend towards hyperopia with time is significant at $P < 0.02$ level.

4. Discussion

All previous studies addressing the refractive state of sharks have concluded they are generally hyperopic with little evidence of accommodative ability, with the exception of an accommodative response under anesthesia in the nurse shark (Sivak & Gilbert, 1976). In the lemon shark, two previous studies of refractive state reported this species to be hyperopic and lacking accommodation (Sivak, 1974; Hueter 1991). However, every previous study of shark refractive state has been conducted on restrained animals. Ours is the first investigation on unrestrained, unanesthetized, free-swimming sharks and our results document that unrestrained lemon sharks are approximately emmetropic, but restrained lemon sharks become significantly hyperopic. We also found evidence of accommodative changes in the eyes of unrestrained lemon sharks, unlike previous studies using restrained animals.

Our results indicate that the normal, resting refractive state of the free-swimming lemon shark is emmetropia, not hyperopia, and the animals are capable of voluntary accommodative excursions from at least $+1.5$ D hyperopia to approximately -1.5 D myopia.

Our finding of hyperopia in restrained lemon sharks is consistent with the calculation of refractive state in a schematic eye for this species (Hueter 1980, 1991; Hueter & Gruber, 1982). That schematic eye was constructed based on a combination of techniques, all of which utilized restrained animals. The model's resulting refractive state was $+2.76$ D, which is in the mid-range of the hyperopias of restrained sharks we measured in this study. The schematic eye, therefore, most likely applies to a juvenile lemon shark under conditions of restraint rather than in a natural, resting state for the eye.

These findings, furthermore, have implications for understanding the accommodative mechanism in elasmobranchs. The Franz (1931) model of elasmobranch accommodation proposed that contraction of the protractor lentis muscle (pseudocampanule), which is a ventral papillary extension of the ciliary body and is attached to the ventral side of the lens, draws the lens towards the cornea and away from the retina, thereby making the eye less hyperopic. If this model is correct, then the eyes of unrestrained, free-swimming lemon sharks would be in a constantly accommodated state; that is, the protractor lentis would be contracted to make the eye emmetropic under normal conditions. With restraint, whether in tonic immobility or not, the intraocular muscle would relax, making the eye hyperopic. The same response would be expected under anesthesia, which is what has been found for accommodation in anesthetized nurse sharks (Sivak & Gilbert, 1976). Several aspects of this mechanism appear to be problematic, notably a constantly accommodated condition during the eye's 'resting' state. Further research into the elasmobranch accommodative mechanism is required to resolve this issue.

Interestingly, the only shark species previously showing any accommodative ability under experimental conditions of restraint is the nurse shark (Sivak & Gilbert, 1976), which is the most benthic (bottom-dwelling) and sedentary of all the species studied by previous investigators. Nurse sharks also are the species used most often in laboratory studies on sharks, due primarily to their unusual adaptability to limited space and handling, and it is plausible that nurse sharks experience less physiological effects from temporary restraint than do the other species of sharks tested. While relatively little is known about stress responses in elasmobranchs, studies have demonstrated significant physiological changes in various shark species subjected to the stresses and exhaustive exercise associated with handling and restraint (Cliff & Thurman, 1984; Jones & Andrews, 1990; Smith, 1992).

Similar to our findings in restrained lemon sharks, owls and kiwis have been reported to assume hyperopic foci with restraint (Murphy & Howland, 1983; Sivak & Howland, 1987). Taken together, these studies point to the value of obtaining measurements of refractive state in unrestrained animals. Such values are more likely to represent accurately the resting refractive state of the eye. It is possible that the hyperopic refractions and loss of detectable accommodation reported in many vertebrate species are in actuality an artifact associated with restraint and/or anesthesia.

Acknowledgements

Portions of this work were supported by NOAA/NMFS Grant NA67FM0199 to REH, NIH Grant EY 02294 to HCH, and by Mote Marine Laboratory.

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